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THE EFFECT OF ANTIOXIDANTS AND DIETARY RESTRICTION ON MORTALITY CURVES

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ABSTRACT

The full exploitation of information contained in mortality curves offers a tool for direct verification of theories of aging. We have analyzed the behavior of mortality curves for middle and high age groups and have proposed a mathematical model of mortality correlated with the rate of aging . The model offers an explanation for the mutual relationships between mortality curves and suggests potential methodologies for determining experimental modification of the rate of aging.

The applicability of this theory is demonstrated by analysis of the changes in mortality as it is influenced by dietary antioxidants or by a calorie restricted diet. Survival data taken from a variety of studies were used as primary information, with parameters of mortality curves being determined by computer–assisted analysis of the curves. These analyses support the hypothesis that a dominant role for free radicals exists in the control of aging in Drosophila. However, in mammals – mice and rats, the effects of antioxidants as well as caloric restriction on mortality curves do not indicate that these treatments alter the rates of aging.

INTRODUCTION

The existence of reliable verification methodologies in gerontology is crucial for future progress in this science. In spite of considerable effort, the problem of verification of hypotheses and validation of theories has not been fully resolved. This has resulted in ambiguity in interpretation of experimental data and in the parallel existence of multiple theories of aging. The first step in the search for verification methodologies in gerontology is to precisely define aging. Numerous definitions of aging have been suggested (Strehler, 1977; Viidik, 1982; Harman, 1994). The essence of the definitions is as follows: Aging is the process of adverse age-related changes in organisms leading to an increased risk of death. With this definition, the criteria for verification of investigation methods should be based on analysis of the "adverse changes" in the functional state of organisms and on analysis of parameters describing the "increased risk of death". Adverse changes in the functional state of an organism may be quantified by measurement of markers of aging, or by measurement of batteries of markers (Dean, 1988; Balin, 1994). Although the benefits of biological age determination are indubitable from many standpoints, the methods currently used to determine biological change are virtually all indirect. In addition, the results are extensively influenced by individual variation and burdened by methodological errors. The risk of death may be quantified by parameters derived from mortality, or survival curves. In comparison with the measurement of biomakers of aging, information derived from mortality or survival curves is more direct, as death is the ultimate and conclusive result of aging. The extent of experimental error is also considerably lower in this methodology. On the other hand, the commonly used parameters of survival (mean and medium life span) are also indirect, as the influence of age-irrelevant factors can not be excluded. The maximum life span is another parameter of survival. In this case, the connection with basic mechanisms of aging seems to be more direct. Unfortunately, the precise determination of this parameter is difficult and problematic. We believe that a more comprehensive analysis of relationships between mortality curves is possible, may lead to the full exploitation of information contained in mortality curves, and may thus contribute to the search for direct methodologies for verification of theories of aging (Doubal and Klemera, 1990, 1997).

Mortality Curves in the Gompertz Period of Life

Exponential growth of mortality with age was first postulated by Benjamin Gompertz (1825) early in the 1800s:

$$R(t) = R_o \exp[(k\,(t-t_o)],$$

where R_o is initial mortality, and k is the exponent (for semilogarithmic representation, k is the slope of a Gompertz straight line).Several decades later Makeham (1860) proposed a modified formula respecting non-gompertzian (e.g. catastrophic) causes of death.

$$R(t) = R_o\, exp[(k(t-t_o)] + M,$$

where M is Makeham´s coefficient to account for the existence of age-irrelevant causes of death.

The Gompertz law of exponential growth of mortality holds true today, with very low dispersion of demographic data for human populations in industrial countries for a substantial part of the adult period of life (Riggs, 1990). The Makeham´s coefficient (M) is, as a rule, negligible during the period from 35 to 85 years of age. The Gompertz law holds true as well for the majority of laboratory mammals and even for many other species of non-laboratory animals. Significant deviations from a Gompertz course of mortality naturally appear in the first

period of life before adulthood (1) and, rather surprisingly, in very advanced age (Riggs, 1990; Klemera and Doubal, 1997).

Strehler in his book Time, *Cells and Aging* (1977) suggested the existence of an inverse correlation between initial mortality R_o and the slope k of mortality curves in human populations living in different environmental conditions. In fact, the mortality curves of human populations seem to be directed roughly to one point and have a "convergent" character (Figure 1).

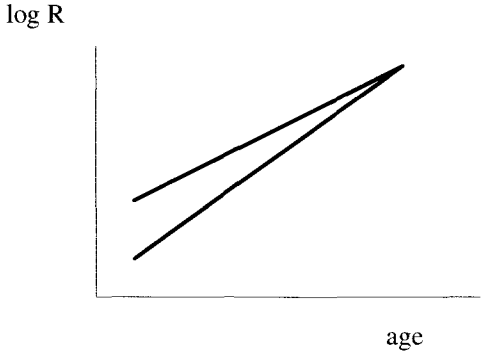

Fig. 1. Convergent mortality curves. This relationship between mortality curves is typical for human populations living in different environmental conditions

The convergent relationship between mortality curves is not a general rule. In papers on experiments with laboratory animals it is possible to find various types of mutual positions for mortality curves. The extreme case seems to be exemplified by the divergent mortality curves (Figure 2) reported for Drosophila living under different temperatures (Bagci and Bozcuk, 1997), or for senescence accelerated mice (Takeda et al., 1997).

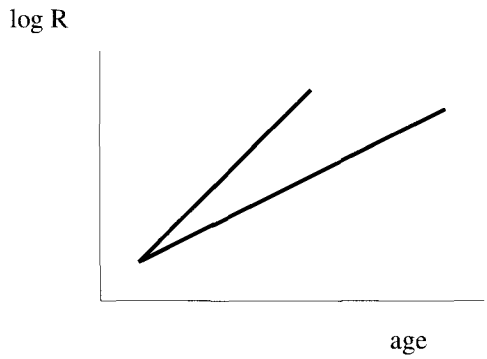

Fig 2. A divergent type of mortality curve

In previous papers we proposed a mathematical model of mortality as a mechanism for analysis of the rate of aging (Doubal and Klemera, 1990, 1997). The model offers an explanation of mutual relationships between mortality curves and suggests a methodology for revealing experimental modifications of the rate of aging. A summary of the conclusions of that model is as follows:

1. Changes exclusively in the slope of the Gompertz line can be caused entirely by changes of "speed" of the pace of aging, i.e. by affecting the aging rate.
2. Vertical displacements of the initial point of the Gompertz line are not caused by altering the rate of aging determinants.
3. The point of intersection of convergent Gompertz lines does not depend on changes of environmental parameters but strongly depends on the parameters of determinants of the aging rate. Hence, influences expressed in convergent Gompertz lines with a common intersection point do not affect the aging rate.
4. The crossing type of Gompertz lines, with the intersecting point in the middle period of life, indicates changes in determinants of the aging rate combined with interventions that affect other characteristics of the organism unrelated to the rate of aging.

Mortality Curves in Advanced Age

Mortality curves cease to follow the Gompertz law in populations of people at an advanced age (Riggs, 1990; Comfort, 1958; Doubal, 1982; Carey, 1992; Smith, 1994). The increase in mortality in humans gradually decelerates at an age above 90 years and the mortality curve reaches its maximum and then remains roughly constant or even decreases in centenarians. There are two possible explanations of this phenomenon.

The first hypothesis presumes that deviation from the Gompertz law may be caused by heterogeneity of the population. In this case, it is presumed that individuals in the population differ in their characteristics of aging. The individuals with a greater risk of death gradually decrease in the population and the older population thus consists of more "age-resistant" individuals. A computer simulation of the changes in mortality of heterogeneous populations, performed in our laboratory, revealed a possible structure of the population with non-Gompertz (and roughly constant) mortality in advanced age. The structure of such a population, however, seems to be rather exotic. Such hypothetical populations should consist of at least two subpopulations with distinctly different mortalities. The relative size of the "longer lived" subpopulation should be about 1 % of the dominant "shorter lived" population.

The second hypothesis is based on the presumption that the population is not significantly heterogeneous. In this case, the course of mortality curves is the result of essential changes in the fundamental mechanisms of aging in an older population. Provided the growth of mortality really reflects the timing of aging, the hypothetical determinant of the rate of aging apparently ceases to count time at this period of life. Consequently, analysis of the course of mortality curves in advanced age may potentially lead to information on the basic mechanisms of aging.

Applications

The theory of behavior of mortality curves in the Gompertz period of life suggests a possible tool for determining

whether the timing of the process of aging would be changed by factors of the experiments. In this sense, the theory offers a direct method of hypothesis verification. The practical application of this theory depends on the reliability and availability of experimental or demographic data. In experimental gerontology the availability of mortality curves represents a considerable obstacle, as direct calculations of mortality require large experimental groups. This problem can be eliminated by computing the parameters of gompertzian mortality curves from available survival curves (Doubal and Klemera, 1997).

To demonstrate the applicability of this theory, we analyzed data on the survival of mice (Harman, 1994) and rats (Pieri, 1991) treated with antioxidants in their food, and food restricted mice (Barrows et al., 1978; Pierpaoli et al., 1995; Boxebaum, 1991; Harman, 1969; Ingram, 1988; Cheney et al., 1980; Witt et al., 1988) and rats (Pieri et al., 1990; Everitt et al., 1982). Further, we analyzed data on the survival of Drosophila melanogaster which had antioxidants added to the diet (Fleming et al., 1981; Massie et al., 1991). Application of the methods for transformation of survival curves (Doubal and Klemera, 1997), and use of a special computer program for evaluation and comparison of survival curves (Klemera and Doubal, in press), allowed us to determine the parameters R_0 and k of Gompertz mortality curves corresponding to the data on survival and statistical evaluation of differences in the corresponding parameters of survival curves. The results are summarized in Table 1.

TABLE 1

animal	*influence of*	*source*	*Change in parameter(s)*	*Gompertz lines*
drosophila	antioxidant	Fleming	$R_0\uparrow$ $k\downarrow$	crossing
drosophila	antioxidant	Fleming	$R_0\uparrow$ $k\downarrow$	crossing
drosophila	antioxidant	Massier	$R_0\uparrow$ $k\downarrow$	crossing
rat	antioxidant	Harman	$R_0\downarrow$ $k\uparrow$	convergent
mouse	antioxidant	Harman	$R_0\downarrow$ $k\uparrow$	convergent
mouse	antioxidant	Harman	$R_0\downarrow$ $k\uparrow$	convergent
rat	restrictive diet	Pieri	$R_0\downarrow$	parallel
rat	restrictive diet	Everitt	$R_0\downarrow$	parallel
mouse	restrictive diet	Ingram	–	parallel ?
mouse	restrictive diet	Weindruch	$R_0\downarrow$	parallel
mouse	restrictive diet	Cheney	$R_0\downarrow$	parallel
mouse	↑ restrictive diet	Witt	$R_0\downarrow$ $k\downarrow$	divergent *
rat	starvation	Everitt	$R_0\uparrow$ $k\to 0$	convergent **
drosophila	temperature	Bagci	$k\downarrow$	divergent
mouse	genetic	Takeda	$k\downarrow$	divergent

Comment. Increment ↑ or decrement ↓ of parameter means: the change was proved at significance level 0.05 or better.

* The level of the diet was gradually increasing in that experiment.

** The starvation caused constant mortality – see text.

DISCUSSION

The addition of antioxidants to their food results in crossing types of mortality curves (Figure 3) for Drosophila. According to the methods for interpretation of the behavior of mortality curves (see above), the antioxidants decelerated aging in these experiments. On the contrary, addition of antioxidants to the diet led to the convergence of

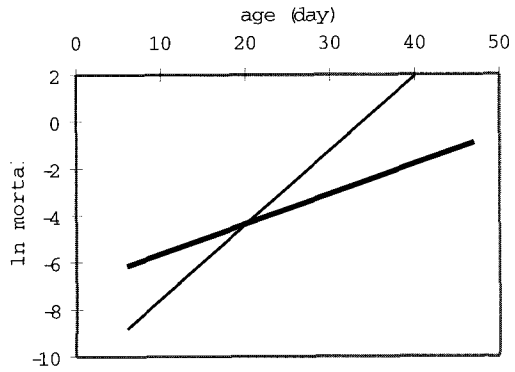

Fig. 3. The effect of antioxidant (ethidium bromide) on mortality of Drosophila melanogaster (Fleming 1981). Thick line – experiment, thin line – control.

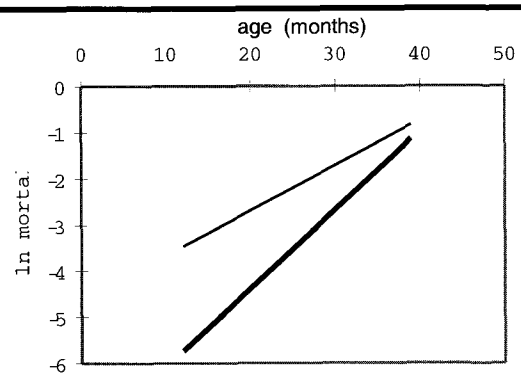

Fig. 4. The effect of antioxidant (MEA) on mortality of the mouse (Harman 1994). Thick line – experiment, thin line – control.

mortality curves in mice and rats (Figure 4). The presence of supplemental antioxidants in these mammals seems not to result in changes in the timing of the aging process, at least in the experiments analyzed here.

The results of analyses of survival data for mice and rats fed a calorie restrictive diet do not provide a uniform pattern (Everitt et al., 1982; Weindruch, 1984). The relationship between Gompertz lines is parallel, sometimes with an unproved tendency to slight divergence or convergence (Figure 5). This relationship does not indicate changes in the aging process. Experiments with strongly food-restricted rats reported by Everitt et al. (1982) represents an extreme situation. Highly restricted animals had high, and almost constant, mortality. The mortality curve is (naturally) convergent in comparison with the control group, and experimental animals did not live sufficiently long to exhibit aging. This situation is reported to be typical for wild animals. These analyses support the idea of a possible role for free radicals in the control of aging of simple organisms. In higher organisms, the effect of free radicals in the experiments analyzed here seems not to be associated with timing of the process of aging. Obviously, more experimental data are necessary for the generalization of these conclusions.

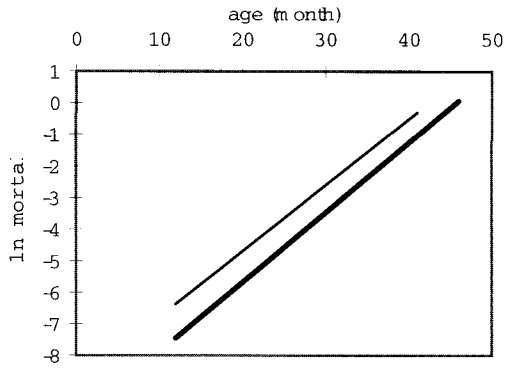

Fig. 5. The effect of a restricted diet on mortality of the mouse (Weindruch 1984). Thick line – experiment, thin line – control.

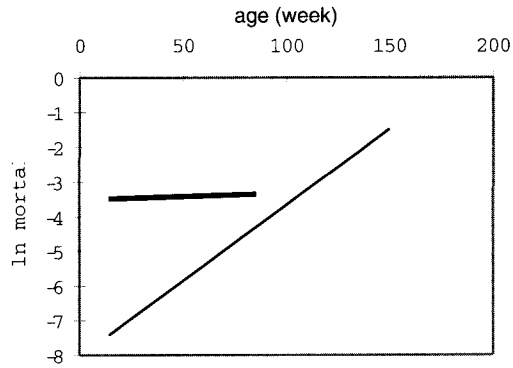

Fig. 6. The effect of extreme starvation on the mortality of rats (Everitt 1982). Thick line – experiment, thin line – control.

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